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## Antibiotic release from bone cement under simulated physiological conditions

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Bone cement loaded with  
clindamycin or fusidic acid in  
addition to gentamicin and  
survival of gentamicin-resistant  
coagulase-negative staphylococcal  
strains in a simulated prosthesis-  
related interfacial gap

## **Chapter 4**

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Chapter 4 is submitted to FEMS Immunology and Medical Microbiology. Hendriks JGE, Neut D, Van Horn JR, Van der Mei HC, Busscher HJ.



## Introduction

Biomaterial-related infections constitute a major threat to the current use of biomaterials.<sup>1</sup> In orthopaedics, use of bone cement loaded with one antibiotic to prevent and treat prosthesis-related infections is widespread, but still controversial. Animal and clinical studies indicate that it is effective,<sup>2-5</sup> but a correlation with the emergence of antibiotic resistance has also been shown.<sup>6-8</sup> The mechanism by which this resistance evolves is not clear and different options have been reported, including mutation,<sup>9</sup> selection<sup>10</sup> and the biofilm mode of growth. In the latter form bacteria may become refractory to antibiotics due to slime formation or slow metabolism,<sup>11,12</sup> although increased resistance of bacteria adherent to the base material of bone cement, polymethylmethacrylate (PMMA),<sup>13</sup> has been shown to persist after adhesion.<sup>14</sup>

A model simulating the *in vivo* interfacial gap,<sup>15</sup> existing between bone cement and bone or prosthesis was developed earlier.<sup>16</sup> High concentrations of antibiotics were achieved in such gaps made in commercially available gentamicin-loaded bone cements. Gentamicin-sensitive bacterial strains were not able to survive in the gap model,<sup>10</sup> whereas in a model with a smaller area to volume ratio, such as the modified Robbins device, gentamicin-sensitive strains could grow on these bone cements.<sup>17</sup> On the other hand, a highly gentamicin-resistant strain (sensitivity > 256 µg/ml) was able to survive in a gap environment, indicating the possibility of selection as a basis for the gentamicin resistance seen after use of gentamicin-loaded bone cement.<sup>10</sup>

Emerging antibiotic resistance in total joint arthroplasty is a threat to current clinical practice and thus new treatment strategies are considered.<sup>18</sup> These encompass more careful prescription of antibiotics<sup>19-21</sup> and combinations of antibiotics.<sup>22-24</sup> The latter has also led to further research in the incorporation of multiple antibiotics in bone cements,<sup>25-29</sup> and recently in a commercially available bone cement (Copal, Biomet Merck, The Netherlands). Studies on combinations of antibiotics included in bone cements using the interfacial gap model could be useful to estimate the clinical value of these combinations, especially for highly gentamicin-resistant strains, not affected by bone cements loaded with gentamicin as a single antibiotic. The aim of this study is therefore to evaluate bacterial killing of highly gentamicin-resistant bacteria in a gap, made in bone cements containing only gentamicin

compared with bone cements in which half of the gentamicin is replaced by the corresponding activity equivalent of clindamycin or fusidic acid.

## Materials and methods

Three experimental bone cements were donated by Johnson & Johnson DePuy CMW (United Kingdom). The first bone cement was loaded with 2.8 w/w% gentamicin sulphate (G), the second with 1.4 w/w% gentamicin sulphate and 1.0 w/w% clindamycin hydrochloride (C) and the last bone cement with 1.4 w/w% gentamicin sulphate and 1.8 w/w% sodium fusidate (F). The method for preparing bone cement blocks with a 200 µm gap is detailed elsewhere.<sup>16</sup> In short, the bone cement dough resulting from mixing the powder and liquid components was applied to a polytetrafluoroethylene (PTFE) mould fitted with 200 µm wide stainless steel strips. After 24 h the blocks were removed from the moulds and the strips. Blocks with superficial surface defects were discarded. Sterile precautions were observed throughout this procedure.

Three coagulase-negative staphylococci (CNS) with a gentamicin sensitivity of > 256 µg/ml (E-test, AB Biodisk, Sweden) were used, all isolated from patients with orthopaedic prosthesis-related infections treated in the University Hospital of Groningen (The Netherlands). All strains were similarly sensitive to clindamycin and fusidic acid, as estimated from the inhibition zones around the relevant bone cement sample in a modified Kirby-Bauer test.<sup>30</sup> The gaps were filled with 6 µl of a 1:10 dilution in Tryptone Soya Broth (TSB, Oxoid, United Kingdom) of a 24 h preculture of the strain in TSB. The inoculated gaps were incubated for 24 h at 37°C, after which the blocks were broken. The gap surfaces were scraped off and the scrapings were resuspended in 9 g/l sodium chloride. After serial dilutions, TSB agar plates were used to quantify the number of colony forming units (cfu) that had survived in the different gaps. Plates were incubated for 7 days to allow detection of slowly growing colonies after antibiotic exposure. A similar serial dilution and plating technique had been used to quantify the inoculum and the percentages of surviving bacteria with respect to the initial inoculum were calculated. All experiments were carried out in triplicate and the differences between the survival percentage in G were compared with that in C and F using Student's t-test for paired samples, since the inoculum size for each bone cement was identical in one run, but not between runs.

Table 4-1. Survival percentages of inocula after 24 h inoculation in a gap in antibiotic-loaded bone cements for the highly gentamicin-resistant bacterial strains used in this study. The p-values as calculated with a two-tailed Student's t-test for paired samples are also given.

Strain	Survival %			p-value	
	G	C	F	G vs. C	G vs. F
CNS 5115	0.154	0.006	0.265	0.09	0.15
CNS 7347	0.008	0.003	0.000	0.42	0.22
CNS 7391	0.106	0.009	0.009	0.19	0.19

G indicates bone cement with 2.8 w/w% gentamicin sulphate

C contains 1.4 w/w% gentamicin sulphate and 1.0 w/w% clindamycin hydrochloride

F contains 1.4 w/w% gentamicin sulphate and 1.8 w/w% sodium fusidate.

## Results

The average inoculum sizes per gap differed per strain and amounted  $3.3 \times 10^6$  cfu for CNS 5115,  $0.8 \times 10^6$  cfu for CNS 7347 and  $3.5 \times 10^6$  cfu for CNS 7391. The percentages of these inocula that survived in the gaps after 24 h of inoculation are presented in Table 4-1. There was a trend of more reduced survival in the bone cement containing gentamicin and clindamycin compared with that containing only gentamicin, but not for the bone cement containing gentamicin and fusidic acid. Taking into account that the results are subject to microbiological variations, the lowest p-value of 0.09 for CNS 5115 in C can be considered to be statistically significant. The relative survival for CNS 7347 was approaching the detection limit in all three bone cements, possibly as a consequence of the four times lower inoculum size. Differences in gentamicin sensitivity could also play a role, although this was  $>256 \mu\text{g/ml}$  for all strains.

## Discussion

Combinations of antibiotics in bone cements have been suggested as an option to counter the emerging antibiotic resistance with the use of only gentamicin. In this study, the survival of highly gentamicin-resistant bacterial strains from prosthesis-related infections was studied in simulated prosthesis-related interfacial gaps in bone cements with gentamicin alone and gentamicin complemented with clindamycin or fusidic acid. The choice of these additional antibiotics can be rationalized. Susceptibility to clindamycin of bacteria adherent to PMMA has not been shown to change due to growth in the adhered state.<sup>31,32</sup> The combination of gentamicin and clindamycin in bone cement has recently been shown to be able to produce a

larger zone of inhibition than bone cements with only gentamicin.<sup>29</sup> Fusidic acid, with its steroid-like structure, is a lipophilic antibiotic that could be applied as a ‘slime buster’.<sup>29</sup> It has good efficacy against staphylococci and good bone penetration and has therefore been suggested as a promising agent against bone infections.<sup>33</sup>

Clindamycin has been reported to have release characteristics from bone cement that are superior to those of gentamicin,<sup>34</sup> but the opposite has also been found.<sup>35</sup> Literature is equally divided as to the release of fusidic acid from bone cement.<sup>25,34,35</sup> The combination of gentamicin and fusidic acid in bone cement was found to have an unexpected short effect, which was ascribed to incompatibility of these compounds in solution due to the acidity of gentamicin in solution.<sup>25</sup> After finding conflicting results between a modified Kirby-Bauer test and a bacterial adhesion assay using a bone cement containing gentamicin and clindamycin, it was suggested that the value of such a bone cement must be proven *in vivo*.<sup>29</sup>

The simulated prosthesis-related interfacial gap model offers an additional *in vitro* test that could be used to estimate the value of antibiotic additions to bone cement.<sup>10</sup> The results of the current study show that all inocula are reduced to less than 0.3 % of their original viability. Although it might be concluded at this stage that the three bone cements used in the current study have adequate bacterial killing, any micro-organism surviving the initial burst release can be expected to lead to a biofilm infection. Highly gentamicin-resistant strains, able to survive for 24 h in a gap environment in gentamicin-loaded bone cement, are only moderately more efficiently killed if half of the gentamicin is replaced with fusidic acid or clindamycin, despite good sensitivity of the selected strains to these antibiotics. In one strain a limited increase in survival was even seen with combined use of fusidic acid and gentamicin. In conclusion, this study shows that use of a combination of antibiotics in bone cement does not uniformly lead to better results than use of gentamicin as a single antibiotic, although the combination of clindamycin and gentamicin showed reduced survival for all three strains.

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